REMARKS

The Specification has been amended to correct a proofreading/typographical error that had resulted in "proteoglycan" being misspelled at page 50, line 17.

Independent claim 31, from which the remaining claims depend directly or indirectly, has been amended to point out with more particularity and clarity the nature and location of MN's cell adhesion site. Claim 31 has been amended to incorporate the subject matter of now cancelled claim 38 that identifies MN's cell adhesion site as being located within MN's proteoglycan-like domain.

Responding to some of the statements made by Examiner during the November 7, 2005 interview, Applicants have further amended claim 31 to emphasize respectfully that MN's cell adhesion site "being within MN's proteoglycan-like domain" consists of an amino acid sequence from SEQ ID NO: 50, which SEQ IS NO: 50 represents the amino acid sequence of MN's proteoglycan-like domain. Support for the proteoglycan-like domain (PG domain) being encoded by **SEQ ID NO: 50** is provided in the Specification at the least at page 19, lines 18-20, which reads: "The extracellular domain contains the **proteoglycan-like domain [aa 53-111 (SEQ ID NO: 50)]** and the carbonic anhydrase (CA) domain [aa 135-391 (SEQ ID NO: 51]." [Emphasis added.]

Independent claim 31 has further been amended to point out with more particularity and clarity that MN's cell adhesion site is

> **closely related or identical to the epitope for MAb M75**, at least two copies of which are located in the 6-fold tandem repeat of 6 amino acids [aa 61-96 (SEQ ID NO: 97)] **in the proteoglycan-like domain of MN protein.**

[Specification, page 62, lines 29-32; emphasis added.] Since MN's cell adhesion site is "**closely related or identical to the epitope for MAb M75 . . .**" [*id.*], Applicants have amended claim 31 for particularity and clarity to indicate that MN's cell adhesion site comprises "an amino acid sequence selected from SEQ ID NOS: 10 AND 98-103." **SEQ ID NOS: 10 and 98-103 are the exemplary amino acid sequences that Applicants consider to represent the MAb M75's epitope as disclosed at page 50, lines 18-24 of the Specification.**

Now amended claim 39 had included SEQ ID NOS: 10 and 98-103 within the group of amino acid sequence that MN's cell adhesion site can comprise. Applicants have reduced the group of amino acid sequences from now amended claim 39 from SEQ ID NOS: 10 and 97-106 to the subset in amended claim 31 of SEQ ID NOS: 10 and 98-103 to define with more particularity and clarity the identity of MN's cell adhesion site as being "**closely related or identical to the epitope for MAb M75 . . .**" [Specification, page 62, lines 29-30; emphasis added.]

SEQ ID NOS: 97, 104, 105 and 106, described at least at page 50, lines 16-18 and lines 25-29, each contain one or more of the exemplary peptides that represent MAb M75's epitope, that is, SEQ ID NOS: 97, 104, 105 and 106 each contain one or more of the SEQ ID NOS: 10 and 98-103. By definition, SEQ ID NOS: 97, 104, 105 and 106 are then MN polypeptides that comprise MN's cell adhesion site and can be useful in the claimed cell adhesion assays, as exemplified by SEQ ID NO: 106 as described in the Specification at least at page 68, lines 1-12.

The Table below shows the close relationship among SEQ ID NOS: 10 and 98-103 and indicates that each of those sequences are within MN's proteoglycan-like domain, whose **amino acid (aa) sequence** [SEQ ID NO: 50] is from aa 53 to aa 111 of Figure 1. The Table further shows the amino acid sequence for SEQ ID NOS: 97, 104, 105 and 106, and the close and/or nested relationships among those and all the sequences in the Table.

TABLE

SEQ ID NO	Amino Acid ("aa") Sequence for the MN Protein as Shown in Figure 1
50	aa 53-111 which is the proteglycan-like domain of the MN protein (Specification, p. 19, lines 15-19, particularly lines 18-19)
10	aa 62-67 (EEDLPS; Specification, page 50, lines 19-20)
97	aa 61-96 "The M75 epitope is considered to be present in at least two copies within the 6X tandem repeat of 6 amino acids [aa 61-96 (SEQ ID NO: 97)] in the proteglycan domain of the MN protein." (Specification, page 50, lines 16-18)
98	aa 61-66 (GEEDLP; Specification, page 50, lines 20-21) aa 79-84 aa 85-90 aa 91-96
99	aa 62-65 (EEDL; Specification, page 50, line 21) aa 80-83 aa 86-89 aa 92-95
100	aa 62-66 (EEDLP; Specification, page 50, lines 21-22) aa 80-84 aa 86-90 aa 92-96
101	aa 63-68 (EDLPSE; Specification, page 50, lines 22-23)
102	aa 62-68 (EEDLPSE; Specification, page 50, line 23)

SEQ ID NO	Amino Acid ("aa") Sequence for the MN Protein as Shown in Figure 1
103	aa 82-87 (DLPGEE; Specification, page 50, line 23-24) aa 88-93
104	aa 51-72 (Specification, page 50, line 27)
105	aa 61-85 (Specification, page 50, line 27)
106	aa 75-98 (Specification, page 50, lines 27-28)

Independent claim 31 has further been amended to point out with more particularity and clarity that either MN protein and/or MN polypeptide that comprise the newly identified cell adhesion site could be used in the cell adhesion assay. The application reads at page 44, lines 19-22:

> A "polypeptide" is a chain of amino acids covalently bound by peptide linkages and is herein considered to be composed of 50 or less amino acids. A "protein" is herein defined to be a polypeptide composed of more than 50 amino acids. The term polypeptide encompasses the terms peptide and oligopeptide.

[Emphasis added.] As described in the Specification at page 68, lines 1-12, the representative MN protein/polypeptide against which the Ph.D-7 phage display peptide library was screened for inhibiting cell adhesion is SEQ ID NO: 106, which is the stretch of amino acids from aa 75 to aa 98 of the MN protein as shown in

Figure 1 of the Specification. By definition, SEQ ID NO: 106 is a MN polypeptide, since it contains less than 50 amino acids. Claim 39 has been amended to point out that SEQ ID NO: 106 is exemplary of MN polypeptides that comprise MN's cell adhesion site, which are useful in the claimed cell adhesion assays.

Applicants respectfully submit that no new matter has been entered by the above amendments to the pending claims, and respectfully request entry of the above amendments and reconsideration of the application as amended.

I. 35 U.S.C. Section 102(b) Rejection

Claims 31-32, 34, 37-39 and 41 stand rejected under 35 U.S.C. Section 102 (b) as "being anticipated by Zavada *et al* (Int. J. Oncology 1997; 10:857-863) . . . for the reasons of record. " [Office Action, Section 4, page 2.] Applicants respectfully traverse, first relying on their arguments in their responses dated June 12, 2003 and June 8, 2005 and also upon the explanations and points made as reported above in the Summary of the November 7 Interview. Applicants respectfully point out Zavada et al. (1997) cannot anticipate or render obvious the claimed methods, since the assay of Zavada et al. (1997) as graphically shown in the accompanying Appendix A, could not identify any molecules that would bind specifically to MN's cell

adhesion site, and teaches away from the identity and location of MN's cell adhesion site.

Applicants respectfully counter the statements in the Final Office Action (dated 09/09/05) at page 3 to the effect that the claims read on the MN fusion protein of Zavada et al. 1997. As explained in the Summary of the November 7 Interview, Applicants respectfully, but vigorously assert that the following statement from the Office Action at page 3 is incorrect: "The limitation of 'whose nucleotide sequence is selected from' is open language and thus reads on a fusion protein comprising SEQ ID NO: 1." As explained below and in the Summary of the November 7 Interview, the language in independent claim 31 concerning nucleotide sequences that encode a MN protein or MN polypeptide, that comprise MN's cell adhesion site, excludes a fusion protein such as the GST-MN fusion protein of Zavada et al. 1997.

The MN protein or MN polypeptide used in the screening assay of Claim 31 "is encoded by a nucleic acid whose nucleotide sequence is selected from the group consisting of" SEQ ID NO: 1 or the closely-related nucleotide sequences of groups (ii) and (iii). The phrase "consisting of" is closed, not open language.

The MN protein or MN polypeptide encoded by SEQ ID NO: 1, the nucleotide sequence of (i) in claim 31, can only have the amino acid sequence encoded by SEQ ID NO: 1, which is MN's

cDNA, which encodes the MN protein shown in Figure 1. SEQ ID NO: 1 encodes that specific MN protein, and a nucleotide sequence consisting of SEQ ID NO: 1 only encodes the MN protein of Figure 1, and cannot encode a MN fusion protein, such as that used in the Zavada et al. 1997 assay.

The nucleotide sequences of (ii) must have 80-90% homology with SEQ ID NO: 1 in order to hybridize under stringent conditions to SEQ ID NO: 1's complement. "Only very closely related nt sequences having a homology of at least 80-90% would hybridize to each other under stringent conditions." [Specification, page 43, lines 6-7.] The nucleotide sequences of (ii) of independent claim 31 must have a homology of at least 80-90% to SEQ ID NO: 1, that is, to MN's cDNA. The nucleotide sequences of (ii) then could not encode any or no significant amount of any non-MN protein.

The fact that the nucleotide sequences of (ii) could not encode a fusion protein, such as the GST-MN fusion protein of Zavada et al. 1997, is supported by the following information concerning SEQ ID NO: 1's low homology to MN's closest nucleic acid relatives. As explained in the instant Specification, MN has been found to be the 9th carbonic anhydrase (CA) isoenzyme. [See, for example, the paragraph bridging pages 3-4 of the Specification.] The MN protein, also known as MN/CA IX or simply as CA IX, is considered more closely homologous to other

CA isoenzymes than to any other proteins. The Specification states at page 43, lines 1-12:

> The MN gene was clearly found to be a novel sequence derived from the human genome. The **overall sequence homology between the cDNA MN sequence and cDNA sequences encoding different CA isoenzymes is in a homology range of 48-50% which is considered by ones in the art to be low.** Therefore, the MN cDNA sequence is not closely related to any CA cDNA sequences.
>
> Only very closely related nt sequences having a homology of at least 80-90% would hybridize to each other under stringent conditions. A sequence comparison of the MN cDNA sequence shown in Figure 1 and a corresponding cDNA of the human carbonic anhydrase II (CA II) showed that there are no stretches of identity between the two sequences that would be long enough to allow for a segment of the CA II cDNA sequence having 25 or more nucleotides to hybridize under stringent hybridization conditions to the MN cDNA or vice versa.

[Emphasis added.]

The nucleotide sequences of (ii) that encode MN protein or MN polypeptide, which comprise MN's cell adhesion site, must have at least 80-90% homology to SEQ ID NO: 1, and could not comprise any or no significant amount of any nucleotide sequence that encodes any non-MN amino acid sequence. Certainly, the nucleotide sequences of (ii) could not comprise nucleotide sequences that encode the GST-MN fusion protein of Zavada et al. 1997. The "GST anchor" of the GST-MN fusion protein contains the 26 kDa C-terminus of glutathione-S-

transferase, approximately half the size of MN protein (58 kDa). [Zavada et al., Int. J. Cancer, 54: 268-274 (1993).] Ones of skill in the art have to realize that the nucleotide sequences of (ii), which must be 80-90% homologous to SEQ ID NO: 1, cannot encode the GST-MN fusion protein of Zavada et al. 1997.

The nucleotide sequences of (iii) "differ from SEQ ID NO: 1 or from the nucleotide sequence of (ii) in codon sequence due to the degeneracy of the genetic code." [Claim 31, last 3 lines.] Again, SEQ ID NO: 1, MN's cDNA, encodes the MN protein of Figure 1 of the Specification, whose amino acid sequence is SEQ ID NO: 2. Said nucleotide sequences of (iii) then either encode the same amino acid sequence as does SEQ ID NO: 1, that is, either SEQ ID NO: 2, or the same amino acid sequences encoded by the nucleotide sequences of (ii). Therefore, the same arguments as detailed above concerning why the nucleotide sequences of (ii) and (iii) can not encode a fusion protein, such as the GST-MN fusion protein of Zavada et al. 1997, apply to the nucleotide sequences of (iii).

Applicants respectfully assert that the Office Action is incorrect in stating at page 3 that "the limitations of groups 'ii' and 'iii' do not adequately define sequences by structure or activity and therefore the sequence as disclosed by Zavada *et al.* meets the limitation of the claimed invention." First the structure of the nucleotide sequences of (ii) and

(iii) are closely related to the specific nucleotide sequence of SEQ ID NO: 1 as explained in detail above. Also, as explained above in detail, the Zavada et al. 1997 GST-MN fusion protein cannot be encoded by the nucleotide sequences of (ii) and (iii).

Secondly, the nucleotide sequences of (ii) and (iii) are functionally defined in the language of independent claim 31:

1. as encoding MN protein or MN polypeptide that comprise MN's cell adhesion site to which vertebrate cells adhere in a cell adhesion assay. If a nucleotide sequence does not encode such a MN protein/polypeptide that so functions in the claimed cell adhesion assay, such a nucleotide sequence could not be included in the (ii) or (iii) nucleotide sequences. Said a determination would only require routine experimentation.

2. The nucleotide sequences of (ii) and (iii) must encode, as indicated at lines 29-32 of claim 31, a MN protein/polypeptide that "is specifically bound by the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which is deposited at the American Type Culture Collection under ATCC No. HB 11128. . . ." Again, only routine experimentation is necessary to make such a determination.

Applicants respectfully conclude on that point that the above quoted statement from the Office Action at page 3 that "the limitations of groups 'ii' and 'iii' do not adequately define sequences by structure or activity and therefore the sequence [encoding GST-MN] as disclosed by Zavada et al meets the limitation of the claimed invention" is clearly mistaken.

Applicants also respectfully correct the following statement from the Office Action at page 3, which allegedly characterizes an argument made by the Applicants:

> Specifically, applicant contends that Zavada *et al*. (1997) taught that an Mab75 bound to a PG domain of the MN protein and therefore not within the cell binding domain of the MN protein as claimed.

Applicants to the contrary have made detailed arguments to the fact that Zavada et al. 1997 teaches incorrectly that the MAb M75 does not specifically bind to MN's cell adhesion site. Zavada et al. 1997 states at page 861, col. 1 under the sub-heading "*Cell adhesion assay*": "Blocking of adsorbed MN protein with an excess of MAb M75 did not abrogate the adhesion of NIH3T3 cells." That statement indicates incorrectly that MAb M75 did not interfere with cells binding to the MN fusion protein used, and inferentially that MAb M75 did not specifically bind to MN's cell adhesion site.

The instant application, as detailed several times in Applicants' earlier responses, corrected that error in Zavada et al. 1997, as in actuality, MAb M75 does specifically bind to MN's cell adhesion site, since MN's cell adhesion site is "closely related or identical to the epitope for MAb M75. . . ." [Specification, page 62, lines 29-30.] Since MAb M75's epitope is known to reside within MN's PG domain, once one knows that the MAb M75 binds specifically to MN's cell adhesion site, one

would know that said site is within MN's PG domain. However, Zavada et al. 1997 inferentially taught the opposite, that is, that the MAb M75 did not specifically bind to MN's cell adhesion site, and therefore taught away from one identifying the location of MN's cell adhesion site being within MN's PG domain, and from MN's cell adhesion site being related, and certainly not closely related, and even more certainly not identical to MAb M75's epitope.

Applicants are respectfully baffled by the following statement from page 4 of the Final Office Action (dated 09/09/05):

> [T]he limitation of the claimed invention have been meet because one of ordinary skill would have been able to identify a compound (i.e. an Mab75 antibody) that does not inhibit the adhesion of cells to the MN protein.

That statement represents the essence of the inoperability of the Zavada et al. 1997 assay. Applicants have pointed out again and again that the MAb M75 is a molecule that inhibits cell binding to MN's cell adhesion site, because MAb M75 binds specifically to MN's cell adhesion site, which is "closely related or identical to the epitope for MAb M75. . . ." [Specification, page 62, lines 29-30; emphasis added.] The claimed methods are designed to identify molecules, such as, MAb M75 which do inhibit adhesion of cells to the MN protein.

Only molecules identified by the claimed methods that do specifically bind to MN's cell adhesion site have utility according to the subject invention. Applicants respectfully submit that the screening methods of the invention can also not be anticipated by Zavada et al. 1997 because 1) only molecules that do bind MN's cell adhesion site have any utility according to the invention; 2) Zavada et al. 1997 tested two compounds (M75 Mab and acetazolamide) but did not identify any compound that does inhibit cell binding; and 3) Zavada et al. 1997 wrongly identified the M75 Mab as a compound that does not inhibit the adhesion of cells to the MN protein.

Critical to the inventive concept underlying the instant claims was the Applicants' identification of the location and nature of MN's cell adhesion site. As explained above, Zavada et al. 1997 only taught away from the location and nature of MN's cell adhesion site.

In claim 31 as amended, a MN protein or MN polypeptide used in the screening assay must be one that "comprises said (cell adhesion) site." Without knowledge of the location of the cell adhesion site of the MN protein, one of skill in the art could not know from Zavada et al. 1997 whether a particular "MN protein or MN polypeptide" encoded by SEQ ID NO: 1 or by nt sequences closely related to SEQ ID NO: 1 could be used to screen for compounds that would inhibit cell binding to the MN

protein (such as the representative MN polypeptide SEQ ID NO: 106, as described in the instant specification), and in fact, would be directed away from choosing a correct MN protein or MN polypeptide for the assay. Assays using an incorrect MN protein or MN polypeptide, that is, that do not comprise MN's cell adhesion site, are by definition inoperable, as explained above and infra.

Since the MAb M75 is approximately the size of the MN protein (infra), one would predict that if the antibody bound the MN protein anywhere near its cell adhesion site, the antibody would certainly inhibit cell binding, but the MAb M75 was reported by Zavada et al. 1997 to not inhibit such cell binding. It was only because of the finding described in the instant specification, that nonapeptides could block both the cell adhesion site and the M75 epitope, that the inventors concluded that MAb M75's epitope and the cell adhesion site were in or close to the same site:

> Naturally, one can argue that the size of MN/CA IX is about the same as of immunoglobulin molecule, and that binding of M75 to its epitope may sterically hinder a different sequence of cell attachment site. This objection has been made unlikely by blocking of both M75 epitope and of cell binding site by nonapeptides 7 + 2 aa. That result strongly suggests that the epitope and the binding site are indeed identical.

[Instant specification, at page 69, line 31 to page 70, line 3.]

The Office Action at page 4 states: "[W]hen the reference relied on expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable." Applicants respectfully submit that Zavada et al. 1997 does not anticipate the location, and certainly not the amino acid sequence of the cell adhesion site of the MN protein, but instead directs one away from the MN location and identity of MN's cell adhesion site; nor does Zavada et al. 1997 anticipate any compound which binds the cell adhesion site of MN protein. Because the experimental design of Zavada et al. 1997 was flawed, one of skill in the art **would not know whether the MN cell binding site was present** within the fusion protein of Zavada et al. 1997, and would not be able to identify a single compound that inhibited cell binding, because a cell binding site was present in the GST portion of the fusion protein. [See graphic at page 2 of the accompanying Appendix A.]

At page 4 of the Final Office Action (dated 09/09/05), the Examiner mistakenly states: "[A]pplicant has not provided any objective evidence [which] would lead one of skill in the art to presume that the reference is in fact not enabling." Applicants respectfully counter that Applicants have provided abundant objective evidence that Zavada et al. 1997 is not enabling, and that the cell adhesion assay of Zavada et al. 1997 is inoperative to identify molecules that would bind

specifically to MN's cell adhesion site. For example, Zavada et al. 1997 does not provide one example of a compound which interferes with cells binding to MN protein and misidentifies the MAb M75 as not interfering with cells binding to MN protein.

Applicants respectfully conclude that Zavada et al. 1997 does not anticipate, nor does it render obvious, the claimed invention. Applicants respectfully request that the Examiner reconsider and withdraw the instant 35 U.S.C. § 102(b) rejection in view of the above remarks.

II. 35 U.S.C. Section 103(a) Rejection

Claims 31-32, 34, 37-39 and 41-42 stand rejected under 35 U.S.C. Section 103(a) "as being obvious over Zavada *et al.* [Int. J. Oncology, 10: 857-863 (1997)] . . . for the reasons of record." [Office Action, Section 6, page 4.] Applicants respectfully traverse, first relying on their arguments in their earlier responses (particularly that dated June 8, 2005) and in the explanations, arguments and points made in the Summary of the November 7 Interview, and in the above response to the 102(b) rejection over the same Zavada et al. 1997 reference. Applicants respectfully submit that Zavada et al. 1997 for the reasons elaborated earlier and above cannot render the subject claims obvious because Zavada et al. 1997 teaches away from the claimed assays.

The subject 103(a) rejection was first set forth in the Office Action mailed from the PTO on February 9, 2005, which Office Action under Section 12 at pages 8-9 states:

> Zavada *et al*. do not specifically or directly teach using human cells in the method outlined in the cited reference of Int. J. Oncology 1997; 10:857-863 so as to anticipate the instantly claimed invention.
>
> However, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use human cells in place of the NIH 3T3 cells used by Zavada *et al*.

Applicants respectfully assert that the graphic and its accompanying explanation of Appendix A as discussed at the November 7, 2005 interview explain why it does not matter whether HeLa cells or mouse NIH3T3 are used in the cell adhesion assay of Zavada et al. 1997, and that the source of the cells used does not change the fundamental error and teaching away of Zavada et al. 1997 from the instantly claimed assays.

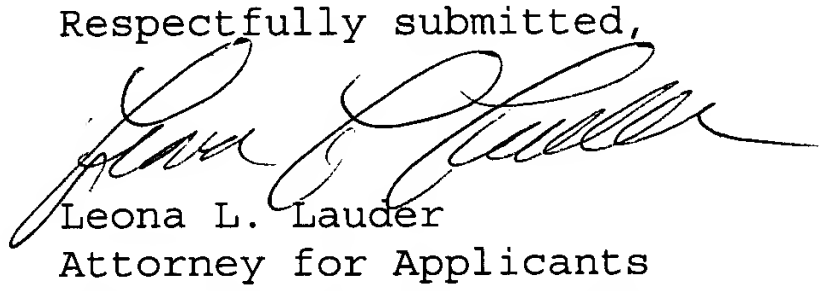
Whatever the cells are that could be envisioned in the Zavada et al. 1997 assay, said cells would still bind to the cell binding site within the GST anchor of the Zavada et al. 1997 fusion protein, and no further information concerning the nature and location of MN's cell binding site would be provided. Applicants' undersigned Attorney respectfully <u>believes</u> that Examiner Yaen expressed that he agreed with that understanding during the November 7 interview.

As explained in detail above and in the response to the 102(b) rejection, Zavada et al. 1997 teaches away from the instantly claimed invention. Zavada et al. 1997 cannot then render the instantly claimed invention obvious. Applicants respectfully request that the Examiner reconsider the instant 103(a) rejection in view of the above remarks, and withdraw this rejection.

CONCLUSION

Applicants respectfully conclude that the claims as amended are in condition for allowance, and earnestly request that the claim amendments be entered, and that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to telephone the undersigned Attorney for Applicants at (415) 981-2034.

Respectfully submitted,



Leona L. Lauder
Attorney for Applicants
Registration No. 30,863

Dated: February 2, 2006

Zavada et al., Int. J. Oncology, 10: 857-863 (1997)

If HeLa cells were substituted for NIH3T3 cells in the cell adhesion assay of Zavada et al., Int. J. Oncology, 10: 857-863 (1997), the M75 monoclonal antibody would still not have blocked the binding of vertebrate (HeLa) cells to the GST-MN fusion protein.

In the diagram, (A) shows the result of M75 Mab incubation with the GST-MN fusion protein before adding NIH3T3 cells (as in Zavada et al. 1997): the NIH3T3 cells appear to not be blocked from binding to the MN cell adhesion site because they bind to a second cell adhesion site located in the GST anchor.[1]

In (B), if MN-positive human HeLa cells are substituted for NIH3T3 cells in the experiment described in Zavada et al. 1997, they would also appear to not be blocked from binding to the MN cell adhesion site because the HeLa cells (although blocked from binding to the MN cell adhesion site on the MN part of the fusion protein) would also bind to the GST cell adhesion site.

---

1 The instant application at page 69, lines 8-13 corrects the error in the 1997 Zavada et al. article by stating:

> There can be no doubt on the specificity of cell attachment to purified MN/CA IX+. It is abrogated by specific MAb M75. . . . **This is a correction to our previous report in Zavada et al., Int. J. Oncol., 10: 857 (1997)** in which we observed that MN/CA IX produced by vaccinia virus vector and **fusion protein GST-MN support cell adhesion**, but we did not realize that **GST anchor itself contains another binding site, which is not blocked by M75**.

[Emphasis added.]

APPENDIX A